Sequential Induction of MHC Antigens on Autochthonous Cells of the Ileum Affected by Crohn's Disease

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Changes were examined in the expression of Class I and II major histocompatibility complex (MHC) antigens by autochthonous cells of the terminal ileum affected by Crohn's disease. The study was based on the analysis of transmural specimens from terminal ileum segments obtained in the course of ileocolectomy for colon cancer and Crohn's disease. Serial sections were immunostained using monoclonal antibodies directed against monomorphic determinants of HLA-A,B,C, DR, DP, DQ, and the invariant chain (Ii) associated with Class II molecules. Compared with the normal state, the only change in Class I antigen expression occurring in Crohn's disease was the induction of HLA-A,B,C antigens in lymphatic endothelium. Changes in Class II antigen expression were more substantial. Enhancement of HLA-DR expression was found in enterocytes; DR induction was observed in glial cells of the visceral nervous plexus and in venular and venous endothelium. HLA-DP and DQ antigens were induced in enterocytes, glial cells, and capillary and venular endothelium, although this induction was restricted to areas of moderate or high inflammatory activity. The tissue distribution of Ii closely resembled that of HLA-DR, although this association was not strict: on the one hand, arterial endothelium contained low amounts of Ii in the absence of DR antigens; on the other hand, glial cells expressed Class II molecules in the absence of Ii. The extent of local enhancement/induction of MHC antigens was positively correlated with the local density of the cellular infiltrate. These data suggest that altered MHC antigen expression by autochthonous structures might be mediated by factors released from the lymphohistiocytic infiltrate, which is itself attracted by an unknown signal. In conjunction with an unknown antigen, the enhanced expression of Class II antigens might trigger an autoaggressive immune response. (Am J Pathol 1987, 129:493–502)

THE MAJOR histocompatibility complex (MHC) is a gene sequence on the short arm of Chromosome 6. It encodes several sets of immunoregulatory molecules. Two classes of them are polymorphic and serve as restriction elements in the cellular immune response. MHC Class I molecules are highly polymorphic transmembranous glycoproteins, noncovalently associated with β_2-microglobulin. In humans they are encoded by three subloci known as HLA-A, B, and C.[1] The expression of these antigens varies greatly among different cell types[2] and is influenced by enhancing stimuli such as interferons[3] and tumor necrosis factor[4] or inhibitory stimuli such as corticosteroids.[5] MHC Class II molecules—likewise polymorphic transmembranous glycoproteins—are products of a gene sequence containing at least three subloci presently designated HLA-DR, DP, and DQ encoding different α and β chains.[6] During intracellu-

lar processing and transport these molecules are associated with an invariant (γ-)chain (Ii)[7] that is not encoded by the MHC but by a gene located on Chromosome 5.[8] On many cell types, HLA-DR, DP, and DQ are differentially expressed,[9,10] again regulated by inductive/enhancing stimuli such as interferons and tumor necrosis factor[11] and inhibitory stimuli such as corticosteroids[5] and prostaglandin E_2.[12] MHC Class I molecules in conjunction with presented antigen are the target structure for autologous antigen-specific cytotoxic T cells, whereas presented

antigen, together with Class II molecules, activate autologous antigen-specific T-helper cells.[13,14]

The etiology of Crohn's disease (CD) is still obscure. Although great efforts have been taken, they have until now all failed to detect a causative infectious agent. However, numerous studies on pathogenesis (recently reviewed by Strober and James[15]) have given rise to the hypothesis of a fundamental antigen-specific immunoregulatory abnormality underlying CD. Following this concept, abnormalities in the local expression of MHC antigens might be expected; and Selby et al[16] have in fact observed an induction of HLA-DR antigens in colonic mucosa of patients with active CD.

This study aims at a detailed analysis of MHC antigen expression in structural cells of the terminal ileum, both in healthy tissue and tissue affected by CD.

Materials and Methods

Patients

An unselected series of 15 patients was chosen for the study. They all had clinically active CD located in the ileum and were admitted to receive surgical treatment. Nine of these patients were men, and 6 were women. The mean age was 33.2 years (range, 18–49 years).

Tissue

Transmural specimens of about 1 sq cm × 0.3 cm were taken from the 15 ileum segments involved by CD immediately after surgical removal. They were quick-frozen in liquid nitrogen and stored at −70 C. In order to study intrapersonal differences in the grade of inflammatory changes, more than one specimen was preserved on 10 occasions; the study is thus based on 38 tissue samples affected by CD. Control specimens of normal terminal ileum were obtained from 5 patients who underwent dextrohemicolectomy for colon cancer (3 men and 2 women with a mean age of 65.2; range, 38–77). Nine serial frozen sections (4–6 μ) from each tissue block were air-dried overnight, subsequently fixed in acetone for 10 minutes, and (immuno)stained immediately or stored at −20 C for a maximum of 20 days.

Reagents

The monoclonal antibodies (MAbs) against MHC antigens and Ii used in this study are listed in Table 1. MAb binding was detected with a polyclonal biotinylated sheep antibody to mouse immunoglobulins and a streptavidin–biotinylated peroxidase complex

Table 1—List of Detected Antigens and of Primary Antibodies Used in This Study

Clone	Specificity	Reference
W6/32	HLA-A,B,C	Barnstable et al[17]
ISCR3	HLA-DR	Watanabe et al[18]
B7/21	HLA-DP	Royston et al[19]
TÜ22	HLA-DQ	Ziegler et al[20]
VIC-Y1	Invariant chain (Ii)	Quaranta et al[21]

(Amersham, High Wycombe, UK). 3-Amino-9-ethylcarbazole (AEC) and N'N-dimethylformamide (DMF) were obtained from Sigma Chemical Co. (St. Louis, Mo).

Immunostaining Procedure

MAbs were applied in appropriate dilutions to the serial sections of each tissue block. The anti-mouse Ig antibody was diluted 1:50 in phosphate-buffered saline, and the streptavidin peroxidase complex 1:100. Incubation times were 1 hour at room temperature for the primary antibody and 30 minutes for the second and third step reagents. Using AEC as the chromogen (0.4 mg/ml in 0.1 M acetate buffer, pH 5.0, with 5% DMF and 0.01% H_2O_2 for 10 minutes), the peroxidase reaction caused an intense red precipitate. The sections were rinsed in tapwater, counterstained with Harris' hematoxylin and mounted with glycerol gelatin.

Controls

Intrinsic positive controls for immunoreactivity in each section were stained interstitial dendritic cells and follicular mantle lymphocytes of the gut-associated lymphoid tissue that indicated the reliability of the reaction and, at the same time, the maximal staining intensity of this individual reaction. Thus, minor day-to-day variations in staining intensity did not affect the evaluation. The staining series from each of the 38 + 5 tissue blocks contained a negative control without the primary reagent. The only staining observed was in granulocytes whose endogenous peroxidase was not blocked (eg, by H_2O_2/methanol). Evaluation of both aspects, inflammatory activity and MHC expression, was made independently by two pathologists experienced in interpretation of immunostaining; discrepancies in estimations were discussed at a double microscope.

Evaluation of Activity of Inflammation

One section from each serial staining was stained with hematoxylin and eosin for characterization of

the lesion on histomorphologic grounds. Because of the irregular distribution and clustering of inflammatory cells, systematic cell countings have not been carried out. Instead, a score was introduced. Inflammation was graded according to the cellular density of immigrated inflammatory cells. Two main categories were defined for immunohistologic evaluation: inflammation of *low* versus inflammation of *high cellular density*. For each tissue sample, this assessment was made on the mucosa, the submucosa, the tunica muscularis, and the subserosa. Because granulomas are formed by locally maturating monocytoid cells and lymphocytes, they were regarded as part of the inflammatory infiltrate. The serosa was excluded from evaluation because changes in antigen expression on mesothelial cells may also have been caused by adhesive peritonitis.

Evaluation of MHC Antigen Expression

Strongly stained dendritic interstitial cells and follicular mantle zone lymphocytes served as internal standards for the assessment of the intensity of reactivity. Other cells were scored as positive (+) when their staining was as intense as that of the reference cells. A marked decrease in intensity was noted as weakly positive (+°). A cell was regarded as negative (−) only when reference cells were stained in the same area. After evaluation of all cells of a given type (eg, capillary endothelial cells) in a given anatomic area (eg, submucosa) a semiquantitative notation was made to characterize the pattern of antigen expression (see Tables 2–4), with ± indicating that stained and unstained subpopulations existed in approximately equal proportions; x>y indicating that pattern x was predominant, and x>>y indicating that pattern y was detected only very infrequently.

Results

Normal Ileum

Enterocytes

Both villus and crypt epithelium expressed strongly HLA-A,B,C antigens in the cytoplasm and on the cytomembrane. There were no differences in antigen density among the different enterocytes. HLA-DR antigens could be demonstrated within the brush-border of villus epithelia, whereas crypt epithelia almost always failed to express them (Figure 1a). HLA-
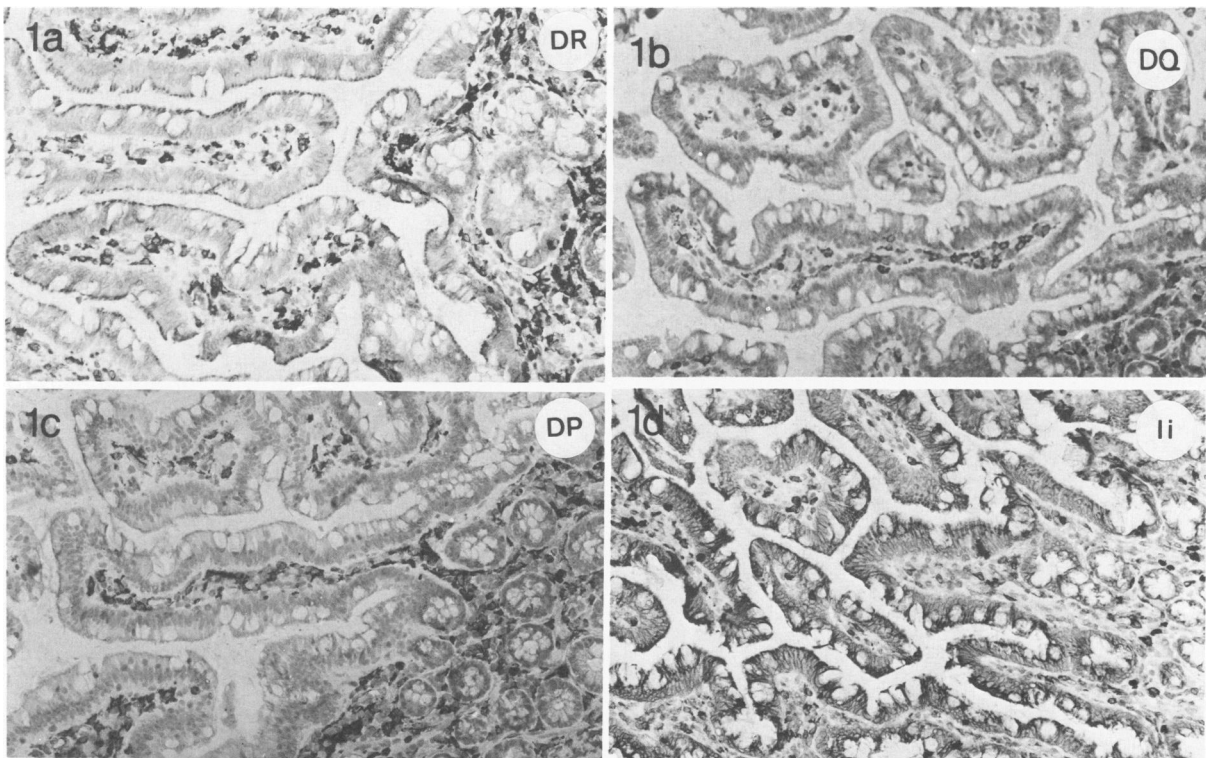

Figure 1—Immunostained serial sections of mucosa of normal ileum. (AEC/hematoxylin, original magnification, ×444)   a—MAb ISCR3 demonstrates HLA-DR antigens on the apex of enterocytes of villi; cells in the lamina propria also strongly express HLA-DR.   b—MAb TÜ22 shows HLA-DQ antigens only in histiocytic cells of the mucosal stroma; enterocytes are DQ-negative throughout.   c—MAb B7/21 shows strong expression of HLA-DP antigens in histiocytic cells; enterocytes are DP-negative throughout.   d—MAb VIC-Y1, recognizing a cytoplasmic epitope on Ii, shows Ii uniformly expressed in villus epithelium; some cells within the villus stroma are stained.

DQ (Figure 1b) and HLA-DP (Figure 1c) antigens were undetectable. The invariant chain showed a distribution pattern very similar to that of HLA-DR antigens. However, Ii was regularly located within the cytoplasm (Figure 1d) which showed the highest antigenic density in the supranuclear region.

Endothelial Cells

HLA-A,B,C antigens were expressed on the entire hemangioendothelium and were uniformly absent from lymphangioendothelium. HLA-DR antigens were inconstantly present on venular and capillary endothelium. HLA-DQ, HLA-DP antigens and the invariant chain were undetectable on/in endothelial cells of normal ileum.

Smooth Muscle Cells

The myocytes of villi, muscularis mucosae, tunica muscularis, and vessel walls were devoid of Class I and Class II antigens and of Ii.

Visceral Nervous System

HLA-A,B,C antigens were strongly expressed on glial cells and nerve fibers of both the submucosal and myenteric plexus. The cytoplasm of ganglion cells, in contrast, was devoid of detectable Class I molecules. The only Class II determinants detectable were HLA-DR antigens that were weakly expressed on nerve fibers of the submucosal plexus. Ii was undetectable.

Crohn's Disease

While we were compiling the data obtained from each of the 38 tissue blocks, it became evident that there were neither intra- nor interpersonal differences in MHC antigen expression that could not be attributed to the degree of *local* inflammatory activity of CD, as evidenced by the density of the *local* cellular infiltrate. Definite deviations from the normal state of

MHC antigen expression were noticed in areas with low density of the cellular infiltrate, but these changes were more pronounced in areas of high cellular density. The results of the semiquantitative evaluation of antigen density in different cell types relative to the local amount of inflammatory cells are given in Tables 2–4.

Enterocytes in CD

Being maximal in the normal tissue specimens, the HLA-A,B,C antigen expression of enterocytes did not change in CD. However, enhancement and/or induction of Class II antigen expression could be observed in diseased areas. HLA-DR antigens became detectable in the supranuclear region of villus epithelia in some areas of low cellular density. They were detected within the entire cytoplasm of villus epithelial cells in all areas of high cellular density, and the villus brush border constantly had high amounts of HLA-DR antigens. In the crypt epithelium, HLA-DR antigens were present in the cytoplasm of cells with the maximum density of antigens at the borders of ulcers, fissures, or areas of ulcerative cryptitis (Figure 2a). Induction of HLA-DP antigens was rare in regions of low density of the cellular infiltrate and appeared as apical membrane staining of the enterocytes. In areas of mucosal damage, on the other hand, there was a strong expression of HLA-DQ (Figure 2b) and HLA-DP (Figure 2c) antigens. In the periphery of these foci, enterocytes showed these antigens in lower density where they were restricted to the apical pole of the cell. The cellular distribution of Ii closely resembled that of with HLA-DR antigen expression (Figure 2d).

Endothelial Cells in CD

In CD, weak and inconstant neoexpression of HLA-A,B,C antigens occurred in dilated lymphatic vessels (Figure 3a). Induction or enhancement of Class II antigen expression was considerable. HLA-

Table 2—MHC Antigen Expression in the Intestinal Epithelium

Antigen	Normal ileum		Crohn's disease, areas of inflammation with low cellular density		Crohn's disease, areas of inflammation with high cellular density	
	Villus	Crypt	Villus	Crypt	Villus	Crypt
HLA-ABC	+	+	+	+	+	+
HLA-DR	+°	->±	+/+°	±	+	+
HLA-DP	–	–	->+°	->>+°	±>–	±>–
HLA-DQ	–	–	->>+°	->>+°	±>–	±>–
Ii	+	±	+	+>>±	+	+

+, intensive staining; +°, weak staining; –, no staining; ±°/±, population consists of (weakly) stained and unstained cells in approximately equal proportions; x>y, pattern x more frequently found among the cases than pattern y; x>>y, pattern x considerably more frequent than pattern y.

Table 3—MHC Antigen Expression in Endothelial Cells of the Ileum

Antigen	Normal ileum					Crohn's disease, areas of inflammation with low cellular density					Crohn's disease, areas of inflammation with high cellular density				
	L	A	V	v	C	L	A	V	v	C	L	A	V	v	C
HLA-ABC	–	+	+	+	+	±°>–	+	+	+	+	±°>–	+	+	+	+
HLA-DR	–	–	–	±	±>+	–	–	–>>±	+>±	+>±	–	–	±	+>>±	+<>±
HLA-DP	–	–	–	–	–	–	–	–	–>>±°	–>>±°	–	–	–	±	±
HLD-DQ	–	–	–	–	–	–	–	–	–	–	–	–	–>>±	–>>±	–>>±
ii	–	±°>–	–	–	–	–	±°>–	–	–	–	–	±°>–	±°>–	±°>–	±°>–

L, lymph vessels; A, arterial vessels; V, veins; v, venules; C, capillaries.
See Table 2 footnote.

Table 4—MHC Antigen Expression in the Intestinal Nervous System

Antigen	Normal ileum				Crohn's disease, areas of inflammation with low cellular density				Crohn's disease, areas of inflammation with high cellular density			
	GPS	NPS	GPM	NPM	GPS	NPS	GPM·	NPM	GPS	NPS	GPM	NPM
HLA-ABC	+	+	+	+	+	+	+	+	+	+	+	+
HLA-DR	–	±°	–	–	–>>±	±	–	–>±	+>±	+	–>±<±	–>±
HLA-DP	–	–	–	–	–<>±	±	–	–	+>±	±	–<>±	±
HLA-DQ	–	–	–	–	–	–>>±°	–	–	–>>±°	–>±°	–	–>>±
ii	–	–	–	–	–	–	–	–	–	–	–	–

GPS, glial cells of plexus submucosus; NPS, nerve fibers of plexus submucosus; GPM, glial cells of plexus myentericus; NPM, nerve fibers of plexus myentericus.
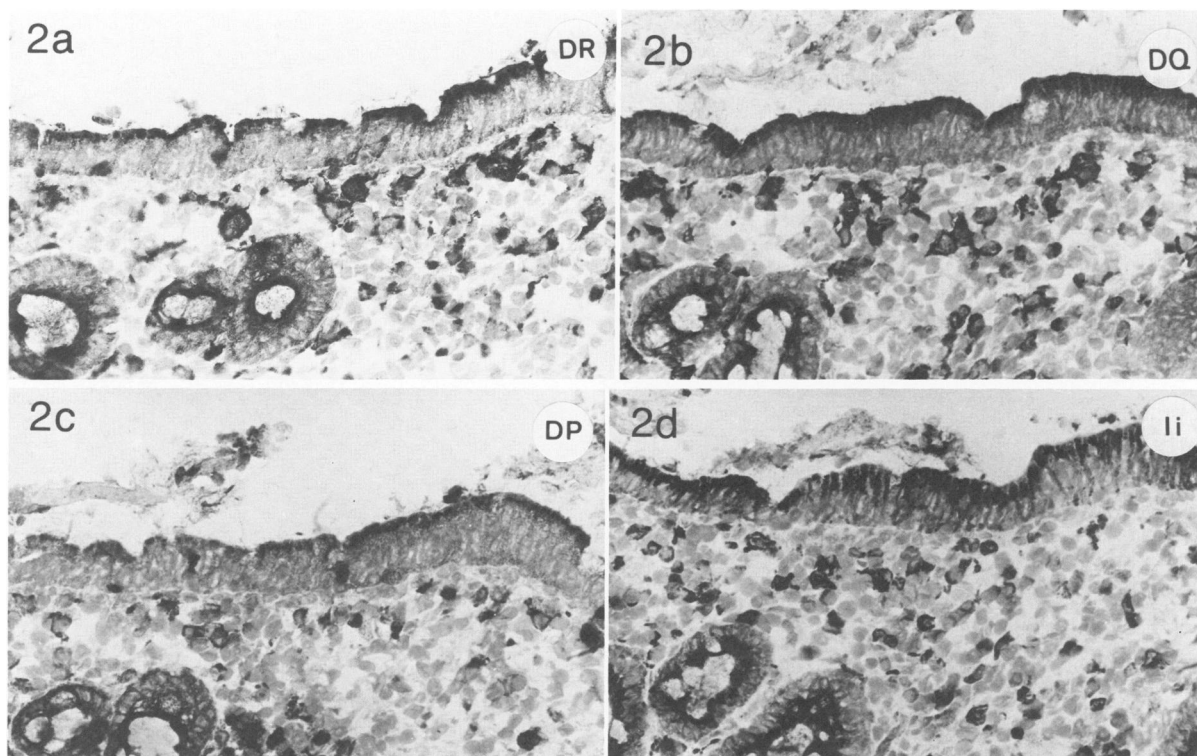See Table 2 footnote.

Figure 2—Immunostained serial section of enteral mucosa bordering a fissure in active CD. (AEC/hematoxylin, original magnification, X888)   a—HLA-DR antigen density is markedly increased at the luminal border of enterocytes, reflecting enhanced HLA-DR expression and induction of HLA-DR molecules in crypt epithelium.   b—HLA-DQ antigens are detectable at the luminal border of enterocytes, including crypt epithelium.   c—HLA-DP antigens are also induced throughout the epithelium. Note that DP-positive cells within the lamina propria are scarce when compared with the number of DR and DQ positives.   d—Compared with normal gut (Figure 1d), enterocytes show enhanced expression of Ii.

DR antigen density correlated in capillaries and venules with the density of the cellular infiltrate. HLA-DR antigens were induced in endothelia of larger veins, while arterial and lymphatic endothelium had none (Figure 3b). Induction of HLA-DP antigens was observed occasionally in capillaries and venular endothelium; the antigen density paralleled the density of the cellular infiltrate. Induction of HLA-DQ antigens was found to be restricted to parts of the endothelium of capillaries and venules in densely infiltrated areas, but this was an inconstant phenomenon. An unexpected finding was the induction of Ii without Class II antigens in endothelial cells of arteries (Figure 3c), even of those located in areas poor in inflammatory cells. On the other hand, HLA-DR antigens were clearly detectable in capillary, venular, and venous endothelium in the absence of detectable Ii.

Smooth Muscle Cells in CD

No changes were observed in expression of either Class I or for Class II molecules by smooth muscle cells in diseased gut.

Visceral Nervous System in CD

in the ileum segments affected by CD, the visceral nervous system was altered structurally. Nerves appeared to be more numerous, and some were considerably enlarged in diameter. Nevertheless, there were no changes in either density or distribution of HLA-A,B,C antigens, but enhancement/induction of Class II molecules could be noted in nerves and glial cells. Even in areas with sparse cellular infiltration, the nerves of the submucosal plexus showed enhanced HLA-DR antigen density and fairly strong induction of HLA-DP molecules. However, this was not observed in all nerves of such an area. In parallel, a subpopulation of glial cells around the ganglion cells expressed HLA-DR and HLA-DP antigens but in considerably lower amounts. Only a faint expression of HLA-DQ antigens could be observed in a very small number of submucosal nerves. All Class II determinants were expressed by nerve structures in the clear absence of Ii. In areas of high density of cellular infiltration, the expression of all three Class II sublocus products was considerably enhanced in the cells
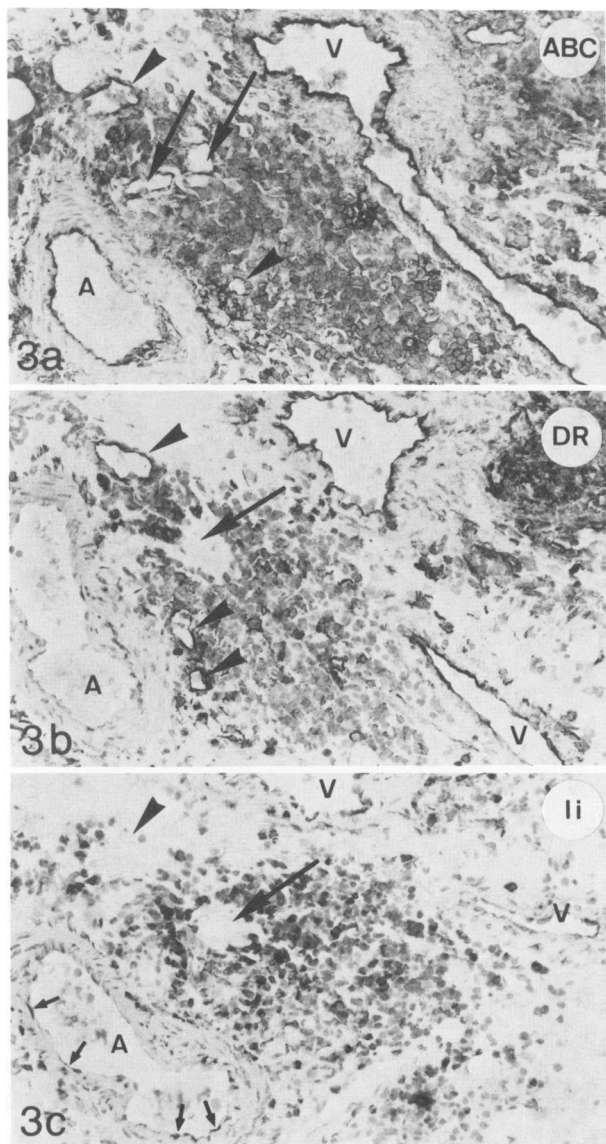
Figure 3—Immunostained serial sections through a densely infiltrated area of the submucosa in active CD comprising an artery (*A*), a larger vein (*V*), venules (*arrowheads*), and some ectatic lymph vessels (*large arrow*). (AEC/hematoxylin, original magnification, ×444)   **a**—MAb W6/32 illustrates the HLA-A,B,C expression. Virtually all cells, even the lymphatic endothelial cells, are stained.   **b**—HLA-DR antigens are expressed in venous and venular endothelium while endothelium of lymphatics and arteries is devoid of detectable DR molecules.   **c**—Ii is weakly expressed in arterial endothelium (*small arrows*) in the absence of detectable Class II antigens (**b**). Venous endothelium contains Ii, while lymphangial endothelium does not.

of the submucosal plexus (eg, HLA-DR, Figure 4a), although Ii could either not be detected within these cells (Figure 4b) or was present in very low amounts. Expression of Class II antigens by the glial cells and nerves of the myenteric plexus was similar, but the extent of Class II antigen induction/enhancement was less pronounced than in the submucosal plexus. Even in densely infiltrated areas, the perikarya of gan-

glion cells were devoid of any of the antigens examined.

In sum, except for smooth muscle cells, lymphatic vessels, and glial cells of the visceral nervous system, HLA-A,B,C antigens were constitutively and strongly expressed in all cell types of the normal ileum, whereas basal DR antigen expression was restricted to a subset of enterocytes and capillary/venular endothelium. In CD, however, and strictly correlated with the density of the lymphohistiocytic infiltrate, HLA-DR antigen expression was found to be enhanced in these structures. In addition, a sequential induction of HLA-DP and DQ antigens occurred in glial cells, nerves, and venous and venular endothelium. This induction was restricted to areas of high density of the inflammatory infiltrate. Concerning the inducibility of the different cell types, the following sequence could be established: villus epithelium → crypt epithelium → lemnocytes → endothelial cells. The distribution pattern of Ii closely resembled that of HLA-DR, but not of DP and DQ antigens. While low amounts of Ii were detectable in arterial endothelium in the absence of HLA-DR, there were large amounts of DR molecules in nerves and glial cells in the absence of Ii.

Discussion

Our immunohistochemical study on ileum affected by CD revealed marked qualitative and quantitative changes in the pattern of MHC antigen expression occurring in autochthonous cells, with the exception of smooth muscle and visceral neurons. A sequential induction/enhancement of Class II antigens was detectable which was clearly correlated with the density of the inflammatory infiltrate.

Because MAbs to nonpolymorphic framework determinants of Class II molecules with sublocus specificity have only recently been characterized, information on differential expression of HLA-DR, DQ, and DP on normal cells and tissue is still scarce. During embryogenesis, sequential expression was found in vascular elements and macrophages in the placenta[22] and in fetal B lymphocytes,[23] in the suggested ordered sequence HLA-DP → DR → DQ. On myeloid progenitor cells, the following sequence was found[24]: HLA-DR → DP → DQ. From those data it was concluded that the three subloci are regulated differentially. Our data strongly support this concept. We found differences in basal (constitutive) Class II antigen expression and induction/enhancement of sublocus products in the sequence HLA-DR → DP → DQ for the authochthonous cells of the ileum affected by CD. This sequence was also observed by
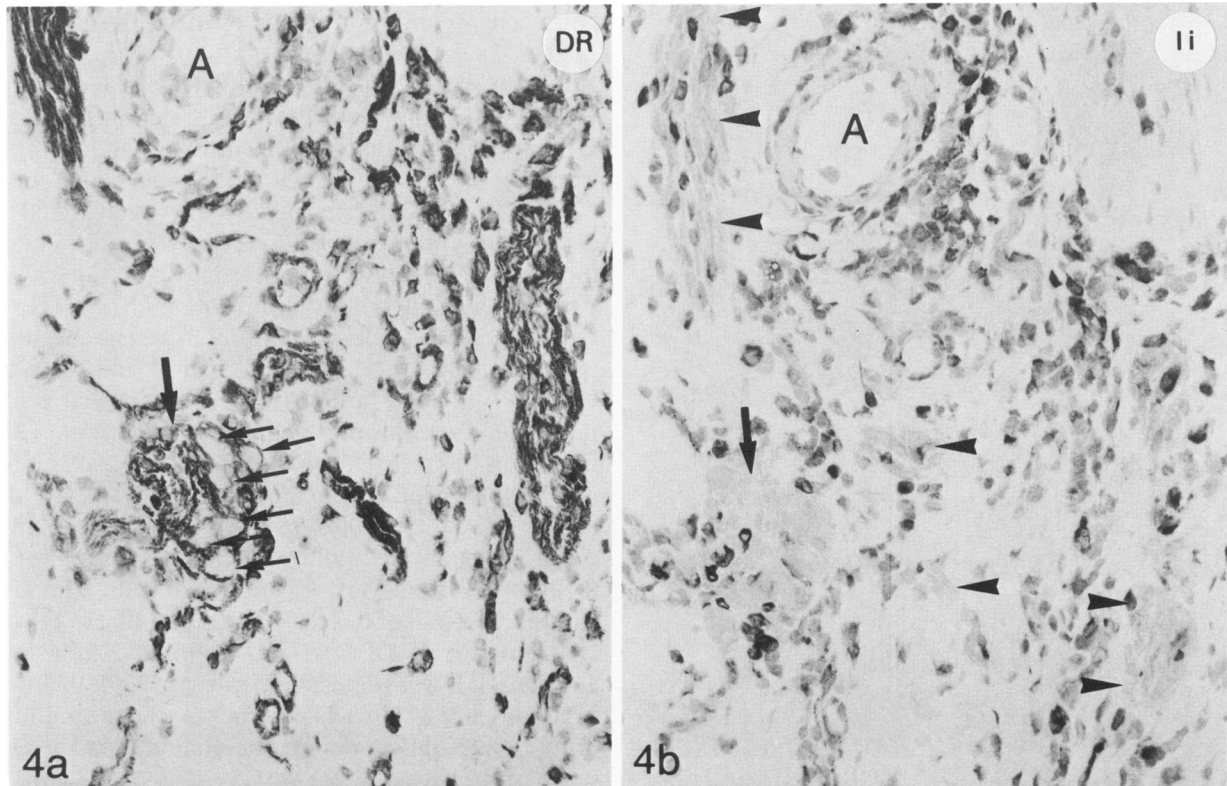
Figure 4—Immunostained serial sections showing the submucosal nerve plexus in an area of high density of inflammatory infiltrate caused by CD. In the depicted area, the density of the inflammatory infiltrate appears low because of some interspersed unreactive adipocytes. (AEC/hematoxylin, original magnification, X750)   **a**—HLA-DR antigens strongly expressed on nerve fibers and glial cells of a submucosal ganglion (*large arrow*). The gangliocytes, in contrast, are devoid of HLA-DR molecules (*small arrows*). A, artery.   **b**—No Ii is detectable in any of the depicted nervous structures. Nerve fibers (*arrowheads*) and the ganglion (*large arrow*) are unstained. The reliability of the staining result is demonstrated by the strongly labeled interspersed dendritic, histiocytic, and lymphocytic cells.

Ghosh et al,[25] who investigated colon mucosa adjacent to colon carcinoma. Presently, these findings cannot be further interpreted, because no clear-cut data on functional differences of the Class II sublocus products are yet available.

Ii, initially found uniquely in Class II antigen immunoprecipitates, was observed to form oligomeric complexes with HLA-DRα and β chains shortly after their synthesis in the endoplasmic reticulum.[7] These complexes were found to be transported to the cytomembrane, where Ii is thought to be either integrated independently from the αβ-dimer[26] or linked to the dimer via the extracytoplasmic region bearing the carboxyl-terminal of Ii.[27] Ii was thus suggested to be a prerogative for posttranslational processing and export of Class II molecules.[28] However, Miller and Germain[29] and Sekaly et al[30] proved that allogeneic cells devoid of endogenous Ii are able to express human Class II antigens on the cytomembrane when transfected with Class II cDNA; cotransfection with Ii cDNA did not enhance this process. The role of Ii has therefore to be reevaluated. Our results clearly demonstrate that in inflamed tissue HLA-DR molecules

are occasionally expressed in the absence of Ii. Whether the cellular HLA-DR$^+$/Ii$^-$ phenotype observed in the visceral nervous plexus and in venous/ venular endothelium is characteristic of certain cell types has to be further elucidated. We could also detect Ii in the absence of any detectable Class II antigen; this was the case in arterial endothelium. Thus, this study furnished clear evidence that the association of Ii and Class II antigen expression is not as close as presently assumed. It may therefore be concluded that Ii has yet another function that is independent from processing of Class II molecules.

A considerable number of studies have shown that induction and/or enhancement of MHC Class I and II antigen expression occurs in a large number of cell types during inflammation, be it infectious, eg,[31,32] allergic, eg,[33] idiopathic, eg,[9,34] or actinogenic. eg,[35] Thus, aberrant MHC antigen expression is by no means indicative of etiology, but rather reflects the degree of the local inflammatory activity.

This argument is in line with results of our studies in mice[36,37] and of human cell culture studies showing that induction/enhancement of MHC antigen ex-

pression can be achieved by applying interferons[11,38] or tumor necrosis factor.[4] On the other hand, prostaglandin E_2[12] and corticosteroids[5,39] each cause a down-regulation, at least of Class II antigens. Some of these factors are secretory products of inflammatory cells.

One of our major findings is that in CD the density of the local cellular infiltrate closely corresponds to the density of Class II antigens in/on autochthonous cells of the diseased ileum. Similar observations have been made in Hashimoto thyroiditis,[34] B hepatitis,[31] and colitis with various causes.[35] This association suggests that MHC antigen expression of autochthonous cells might be induced by soluble factors released by the inflammatory infiltrate.[40] However, this mode of induction is probably not the initial step of the process.

The best known function of MHC molecules is antigen presentation to autologous T cells.[41] These molecules act as nonspecific receptors for antigenic proteins and form a complex with them.[42] According to present view, the complex is internalized, intracellularly processed, retransferred to the cytomembrane, and recognized by the antigen receptor of an antigen-specific T cell.[43] Antigen-complexed Class I molecules will trigger cytotoxic T cells; antigen-complexed Class II molecules will trigger T helper cells. The avidity between the respective T cell and the antigen-presenting target cell may be increased by the T8 molecule binding to an uncomplexed Class I molecule and by the T4 molecule binding to an uncomplexed Class II molecule.[14] Every cell expressing MHC antigens could hence act as an antigen-presenting cell[44] and may thus trigger a cellular immune response. Consequently, inductive mediators such as γ-interferon recruit—in the presence of antigen—antigen presenting cells, leading to a local amplification of target structures for antigen-specific T cells.

Bland and Warren[45] showed that isolated rat enterocytes of the small intestine bound ovalbumin nonspecifically via Class II molecules, which could then stimulate antigen-(ovalbumin-)specific T cells in co-culture. In rats, Class II antigen induction on intestinal villi was proven to be the direct consequence of luminal antigen contact. This induction was apparently not mediated by T cells, because it occurred even in athymic animals.[46] Ciclitira et al[47] reported that gluten challenge of treated celiac patients enhanced/induced HLA-DR antigens in enterocytes within 2 hours. This observation suggests a "primary" Class II antigen-driven process that may also exist in ileal CD: A luminal antigen might bind to the HLA-DR molecules, normally expressed on the brush border of villus enterocytes, and thereby initiate a

change in enterocytic Class II antigen expression. This is one possible way the immunocascade, triggering off inflammatory changes in CD, may start.

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